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Digesta retention patterns of solute and different-sized particles in camelids compared with ruminants and other foregut fermenters

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Abstract

The mean retention time (MRT) of solute or particles in the gastrointestinal tract (GIT) and the forestomach (FS) are crucial determinants of digestive physiology in herbivores. Besides ruminants, camelids are the only herbivores that have evolved rumination as an obligatory physiological process consisting of repeated mastication of large food particles, which requires a particle sorting mechanism in the FS. Differences between camelids and ruminants have hardly been investigated so far. In this study we measured MRTs of solute and differently-sized particles (2, 10, and 20 mm), and the ratio of large-to-small particle MRT, i.e., the selectivity factors ($SF_{10/2mm}$, $SF_{20/2mm}$, $SF_{20/10mm}$), in three camelid species: alpacas (*Vicugna pacos*), llamas (*Llama glama*), and Bactrian camels (*Camelus bactrianus*). The camelid data were compared with literature data from ruminants and non-ruminant foregut fermenters (NRFF). Camelids and ruminants both had higher $SF_{10/2mm}^{FS}$ than NRFF, suggesting convergence in the function of the FS sorting mechanism in contrast to NRFF, in which such a sorting mechanism is absent. The $SF_{20/10mm}^{FS}$ did not differ between ruminants and camelids, indicating that there is a particle size threshold of about 1 cm in both suborders above which particle retention is not increased. Camelids did not differ from ruminants in MRT_{2mm}^{FS} , MRT_{solute}^{FS} and the ratio $MRT_{2mm}^{FS}/MRT_{solute}^{FS}$, but they were more similar to ‘cattle-’ than to ‘moose-type’ ruminants. Camelids had higher $SF_{10/2mm}^{FS}$ and higher $SF_{20/2mm}^{FS}$ than ruminants, indicating a potentially slower particle sorting in camelids than in ruminants, with larger particles being retained longer in relation to small particles.

Key words: digesta kinetics, digesta passage, rumen, digesta washing, selectivity factor

Introduction

The digestive strategy of non-ruminant foregut fermenters has historically been considered ‘ruminant-like’ (e.g. Moir et al. 1954; Bauchop and Martucci 1968), but the process of rumination clearly sets ruminants apart from non-ruminant foregut fermenters (Fritz et al. 2009; Schwarm et al. 2009b; Clauss et al. 2010). True rumination has evolved in only two artiodactyl lineages, the ruminants and the camelids, while sporadic regurgitation and repeated mastication of stomach contents (merycism) has been reported in a variety of mammals such as koala (*Phascolarctos cinereus*) (Logan 2001, 2003), macropods (Moir et al. 1956; Mollison 1960; Barker et al. 1963; Hendrichs 1965), hyrax (*Procavia capensis*) (Hendrichs 1965), capybara (*Hydrochoerus hydrochaeris*) (Lord 1994) and proboscis monkeys (*Nasalis larvatus*) (Matsuda et al. 2011; Matsuda et al. 2014). In contrast to merycism, rumination is an obligatory, regular behavioural and physiological process (Gordon 1968) that is characterised not only by ‘repeated mastication’ but by a density-dependent sorting mechanism in the forestomach (FS) of ruminants and camelids (Lechner-Doll et al. 1991). This mechanism is absent in non-ruminant foregut fermenters (Schwarm et al. 2008, 2009c; Schwarm et al. 2013). In ruminants and camelids, this mechanism ensures that only those particles are ruminated that require further comminution.

While camelids and ruminants both ruminate, several differences set these groups apart: a different chewing motions during rumination (Hendrichs 1965), the different design of the FS (Langer 1988) (Fig. 1), and different FS motility patterns (Heller et al. 1984; Heller et al. 1986b). More generally, camelids have a lower metabolic rate (measured e.g. via oxygen consumption) and a lower food intake than ruminants of comparable body size (Dittmann et al. 2014a). The latter aspect might be related to the longer digesta retention times found in camelids (Heller et al. 1986c). In ruminants, the reticulo-omasal orifice represents a clear demarcation line before which particle sorting takes place in the reticulum, and beyond which only small particles are found (Clauss et al. 2009a; 2009b). In camelids, particle sorting takes

place in compartment C2 that is sometimes also referred to as a ‘reticulum’ (Langer 1988). The connection between the C2 (‘reticulum’) and the C3 (the ‘gastric tube’) is not an orifice but a short tubular canal (Vallenas et al. 1971; Langer 1988). Potentially, this canal does not represent as clear a point of demarcation as the reticulo-omasal orifice in ruminants, because large particles have been found beyond this point in the proximal part of the third compartment (Lechner-Doll and von Engelhardt 1989). These large particles are presumably transported backwards into the C2. Hence, it has been suggested that the sorting mechanism in the FS of camelids is less efficient than in ruminants, and that the emptying of the FS is too slow to allow similarly high relative food intakes as those observed in some ruminant species (Clauss et al. 2010). The retention of solute as well as small and large particle markers has been investigated in camelids (Heller et al. 1986a; Heller et al. 1986c; Lechner-Doll et al. 1990; Cahill and McBride 1995; von Engelhardt et al. 2006b), and a sorting mechanism in the FS, as reflected by longer retention of larger as compared with smaller particles, has been demonstrated (Heller et al. 1986a; Heller et al. 1986c; Lechner-Doll et al. 1990). So far, comparative studies of retention times in ruminants and camelids are lacking. In ruminants, marked differences between species occur with respect to the retention of solute and particle markers (Dittmann et al. 2015). The efficacy of the ruminant sorting mechanism however, is not affected by such species differences (Lechner et al. 2010). Furthermore, while the sorting mechanism of ruminants differentiates between small (< 2 mm) and larger (10 mm) particles, it does not further differentiate between large particle-size classes (10 vs. 20 mm) (Schwarm et al. 2009a; Lechner et al. 2010). To our knowledge, it has not yet been investigated whether the sorting mechanism in the FS of camelids is not only qualitatively, but also quantitatively similar to that in ruminants.

The aim of this study was to assess the retention patterns of solutes and differently sized particles in three camelid species, in order to compare retention times and the sorting mechanism within camelids, and to ruminants and non-ruminant foregut fermenters.

98

99 **Methods**

100 *Animals and husbandry*

101 The measurements were approved by the Cantonal Veterinary Office Zurich and took place
102 under the Swiss Cantonal Animal Experiment Licence no. 142/2011 in the framework of a
103 comprehensive experiment using respiration chambers to determine metabolic rates
104 (Dittmann et al. 2014a) and methane production (Dittmann et al. 2014b) in three camelid
105 species. All experimental animals were adult and included representatives of Bactrian camels
106 (*Camelus bactrianus*, n = 5) and llamas (*Lama glama*, n = 6) kept on a private farm in
107 Switzerland, and alpacas (*Vicugna pacos*, n = 5) kept at Zoo Zurich. Prior to the experiment
108 the animals were acclimated to a diet consisting of lucerne hay provided *ad libitum* and a
109 limited amount of lucerne pellets (for a detailed nutrient analysis of diets, see Dittmann et al.
110 2014b). The pellets eventually made up 53, 33 and 21 % of total dry matter intake (DMI) in
111 alpacas, llamas and Bactrian camels, respectively. In order to ensure comparable *ad libitum*
112 intakes in all species, alpacas received a higher proportion of pellets because the voluntary
113 daily intake of lucerne hay (per unit metabolic body mass) was comparably low in this
114 species. All animals were weighed prior to the experiment. During the experiment, the
115 animals were kept individually on the same diet in separate adjacent indoor pens that allowed
116 visual and acoustic contact. Food intake was determined by weighing diet items offered and
117 the corresponding refusals several times per day for 6 to 7 days. Representative samples of
118 food and refusals were taken and dried at 60°C. Dry matter (DM) content was analysed by
119 drying at 103°C following AOAC no. 942.05 (AOAC 1995). Pens were cleaned on daily basis
120 and animals had unrestricted access to water.

121

122 *Determination of solute and particle retention times*

123 The principle of mean retention time (MRT) measurement is the application (typically as a
124 single pulse-dose) of a non-absorbable marker, the excretion of which over time is then
125 detected by analysing faecal samples for the marker concentration (Warner 1981). To
126 measure MRT of particles and fluid, four markers from the same batch as those used by
127 Lechner et al. (2010) were fed, which are considered representative of four different digesta
128 components: three different sized particle markers based on fibre from grass hay mordanted
129 with Chromium (Cr; < 2 mm), Cerium (Ce; approx. 10 mm), Lanthanum (La; approx. 20
130 mm), and Cobalt ethylene diaminetetracetic acid (Co-EDTA; soluble in water). Markers were
131 prepared according to Udén et al. (1980) and Schwarm et al. (2008; 2009a). Bactrian camels
132 and llamas received all four markers, while alpacas received only Cr- and Ce-mordanted
133 fibres and Co-EDTA; based on our observations of the feeding behaviour of the latter species,
134 we expected reluctance of marker ingestion if too much marker material would have been
135 offered. Prior to the administration of the markers, three faecal samples were collected to
136 determine baseline marker concentrations in each animal. Individuals were then fed the
137 particle markers at 0.1 g kg⁻¹ body mass (BM) each and Co-EDTA at 0.01 g kg⁻¹ BM
138 dissolved in water. Markers were fed in mixture with a small amount of lucerne pellets and
139 were consumed within approximately 30 minutes. The time when the animals had completely
140 ingested the markers was considered 0 h, after which faeces of llamas and Bactrian camels
141 were sampled every 4 h for the first 60 to 84 h after marker application and every 6 h for the
142 remaining time of the 7 days. Faeces of alpacas were sampled every 4 h for the first 2 days
143 after marker application, every 6 h on day 3, every 8 h on day 4, and every 12 h on days 5, 6
144 and 7. Due to differences in facilities and husbandry between species, the sampling protocol
145 differed between species. However, the method used for calculating retention times was
146 independent of sampling intervals, as demonstrated by Van Weyenberg et al. (2006). All
147 samples were immediately oven-dried at 60°C and later ground to 0.75 mm. Marker analysis
148 was performed in a similar way as in previous studies (Frei et al. 2015). For wet ashing we

heated samples with 4 ml nitric acid (HNO₃) and 2 ml hydrogen peroxide with the microwave MLS 'START 1500' (MLS GmbH, Leutkich, Germany). Temperature was increased over 15 min to 170°C, and over 20 min to 200°C, then held at 200°C for 5 min. The wave-length was 12.25 cm and the frequency 2.45 GHz. Determination of Co, Cr, Ce and La in the sample digests was performed using an inductively coupled plasma optical emission spectrometer (model Optima 8000, Perkin Elmer, Rodgau, Germany). Sample introduction was carried out by using a peristaltic pump connected to a Meinhard nebulizer with a cyclon spray chamber. The measured spectral element lines were: Co: 228.616 nm; Cr: 267.716; Ce: 413.764 nm; La: 398.852 nm. The RF power was set to 1400 W, the plasma gas was 8 L argon min⁻¹, whereas the nebulizer gas was 0.6 L argon min⁻¹. Values were corrected for the individual baseline concentrations prior to the marker application. To avoid an artificial increase in MRT by infinite excretion curves due to variation in baseline concentrations, values below 1 % of the maximum concentration of a marker in the excretion curve were set to zero (adapted from Bruining and Bosch 1992).

We estimated MRT in the gastrointestinal tract (GIT) by an algebraic equation, and the MRT of the solute marker in the forestomach using the descending part of the marker excretion curve, following published procedures. MRT GIT was calculated according to Thielemans et al. (1978) as

$$\text{MRT GIT} = \sum (t_i \times dt \times c_i) / \sum (dt \times c_i)$$

where t_i = time after marker application in h determined as the midpoint between two sampling intervals, dt = time interval represented by the marker concentration calculated as $((t_{i+1} - t_i) + (t_i - t_{i-1}))/2$, and c_i = faecal marker concentration at t_i in mg kg⁻¹ DM. In contrast to equations that calculate MRT GIT without considering the time interval dt (Blaxter et al. 1956; Warner 1981), this equation has the advantage that the sampling frequency has no influence on the calculated MRT result (Van Weyenberg et al. 2006).

The mean retention time of the solute marker in the FS (MRT_{soluteFS}) was calculated by estimating the rate constant of the descending part of the marker excretion curve using an exponential equation according to Lechner-Doll et al. (1990) as

$$y = A \times e^{-k \times t}$$

where y = faecal marker concentration at time t in mg kg^{-1} DM, A = constant, k = the rate constant of the descending part of the excretion curve in h^{-1} , and t = time after marker application in h. According to Hungate (1966), the reciprocal value of k represents the MRT within the compartment characterised by k . This approach therefore assumes that the forestomach is the major mixing compartment in the camelid GIT. Based on the assumption that fluid and particles do not differ in passage characteristics distal to the FS (empirically confirmed in ruminants by Grovum and Williams 1973; Kaske and Groth 1997; Mambrini and Peyraud 1997), $MRT_{\text{particleFS}}$ is calculated as

$$MRT_{\text{particleFS}} = MRT_{\text{particleGIT}} - (MRT_{\text{soluteGIT}} - MRT_{\text{soluteFS}}).$$

The selectivity factor (SF) is defined as the ratio of two MRTs, either particle to solute or large to small particles. It was calculated for both total GIT and the FS, and for the small particle marker MRTs to solute MRT (Cr:Co), and for larger to smaller particle MRTs (Ce:Cr, La:Cr, La:Ce).

Comparative literature

Data on the retention of comparable passage markers obtained in various camelids, ruminants and non-ruminant foregut fermenters (NRFF) were collected from the literature. Data on ruminant $MRT_{2\text{mm}}$ and MRT_{solute} are the same as provided in the Supplementary Table of Dittmann et al. (2015). Data sources of 10 mm and 20 mm particle markers from ruminants, camelids and NRFF are presented in Table 1. For the dataset on $MRT_{2\text{mm}}$ and MRT_{solute} we classified the ruminant species as ‘cattle-’ or ‘moose-type’, based on their $SF_{2\text{mm/soluteFS}}$, because ‘cattle-type’ ruminants are defined as having comparatively shorter solute retention

times in the reticulorumen, and thereby higher $SF_{2mm/solute}FS$ values, than ‘moose-type’ ruminants (Clauss et al. 2010).

For NRFF, no data were available for large (20 mm) particle markers. Because data were available from many different species for the solute and small particle (2 mm) markers, the data incorporated in analyses with respect to these markers were averaged per species. Species means for all measures were first calculated as an average per source, and then as mean of all source averages. In total, we collated data from 32 ruminant species (consisting of 13 ‘moose’ and 19 ‘cattle-type’ species), four camelid species and seven non-ruminant foregut fermenter species. For the datasets including 10mm and 20mm particle markers, fewer measurements were available, and therefore analyses were performed with data from individual animals, not species means, and without PGLS analyses (see below).

Statistical evaluation

The relative dry matter intake (rDMI) was calculated using an exponent of $BM^{0.85}$, following Müller et al. (2013). This approach was supported by the data obtained from the camelids investigated in this study, in which DMI scaled at $BM^{0.85}$ (95%CI: 0.75; 0.94). Data from species investigated in the present study were tested for normal distribution by applying a Shapiro-Wilk test, based on which we used ANOVAs for comparison of retention times between and within species, followed by pair-wise Tukey HSD post hoc tests. Data from Bactrian camels were compared with literature data from dromedaries (Lechner-Doll et al. 1990), by applying unpaired two tailed t-tests. All statistical tests were carried out in R 2.15.0 (R Development Core Team 2012) using the packages *ape* (Paradis et al. 2004), *caper* (Orme et al. 2010), and *nlme* (Pinheiro et al. 2011).

Correlations including data from species investigated in the present study and literature data from other herbivores were investigated by applying general least squares (GLS) models with MRT, SF or DMI as dependent variable and BM or rDMI as independent variables. In

the GLSs herbivore type (camelid, ruminant [either as such or separated into ‘moose-’ and ‘cattle-type’] or NRFF) was added as a cofactor. For each model, we tested the interaction between the independent variable and the cofactor. This interaction was removed from the model when not significant. Additionally, to investigate differences in relationships between large and small particle markers between herbivore types, we applied GLSs with large particle markers as dependent and small particles markers as independent variables, and with herbivore type as a cofactor (and interactions of the latter two). The respective SFs were tested for differences between herbivore types by applying ANOVA or Kruskal-Wallis tests, followed by Tukey HSD post hoc tests or non-parametric pair-wise tests as means for multiple comparisons (R function *kruskalmc*).

Species cannot be considered independent units, as they share an evolutionary history which means that similarities between species might only be an artefact of their ancestry (Felsenstein 1985). This lack of independence violates basic assumptions of many statistical tests, which is why we accounted for phylogeny by applying Phylogenetic Generalised Least Squares (PGLS) analyses. Data were linked to a supertree of extant mammals (Bininda-Emonds et al. 2007, 2008), for the same models investigated by GLS in the dataset for MRT_{2mm} for which values of many different species were available, without the inclusion of herbivore type as a cofactor. The value of the phylogenetic signal (λ) (Pagel 1999), which can be considered a measure of the phylogenetic structure in the dataset, was estimated with maximum likelihood (Revell 2010), using the PGLS command from the package *caper* (Orme et al. 2010). Additionally, Akaike’s information criterion (AIC) for the models was determined by using the R function AIC to determine which model has the better fit. Significance levels were set to $\alpha = 0.05$, with values between 0.05 and 0.10 considered as trends.

Results

Differences between camelid species

Marker elimination curves for the three species indicated a typical sequence in marker elimination peaks, with the solute marker being eliminated first, followed by the small particle marker, and then by the two large particle markers (Fig. 2). In the two species (camels and llamas) where three particle markers had been applied, the MRTs (both in GIT and FS) of the two large particle markers did not differ from each other ($P > 0.99$ and $P > 0.79$, respectively). All other MRTs, in GIT and FS, differed significantly between each other within each species (camels: $P < 0.001$; llamas: $P < 0.001$; alpacas: $P < 0.023$) (Tables 2 and 3).

In general, there were no significant differences in retention times of the different markers between species; only llamas had shorter MRT_{20mm} GIT and FS than Bactrian camels ($P < 0.036$). $SF_{10/2mm}$ GIT and FS were lower in alpacas than in llamas ($P < 0.041$) and Bactrian camels ($P < 0.011$).

Comparing our measurements of the large camelid, the Bactrian camel, to literature data from dromedaries (Lechner-Doll et al. 1990), revealed longer MRTs for all markers in the GIT ($P = 0.000 - 0.002$), shorter MRT_{solute} FS ($P = 0.002$) and a trend towards shorter MRT_{2mm} FS ($P = 0.071$) in dromedaries (Tables 2 and 3). Only MRT_{20mm} FS did not differ between the two species ($P = 0.62$). The SFs in the GIT were similar in the two camel species ($SF_{2mm/solute}$ GIT: $P = 0.73$; $SF_{20/2mm}$ GIT: $P = 0.99$), whereas $SF_{2mm/solute}$ FS and $SF_{20/2mm}$ FS were each higher in dromedaries than in Bactrian camels (both $P < 0.001$).

Comparisons with literature data from ruminants: absolute MRTs

When relating combined data from ruminants and camelids on MRT_{2mm} and MRT_{solute} , both for GIT and FS, to body mass, there were no significant interactions ($P > 0.37$) between herbivore type and BM. There were no significant differences ($P = 0.10 - 0.94$) between camelids and ruminants, or between camelids, ‘cattle-’ and ‘moose-type’ ruminants in these

models. Camelid values were within the range reported for ruminants (Fig. 3). $MRT_{2mm}GIT$ and $MRT_{solute}GIT$ were related to BM in GLS ($P < 0.035$; scaling exponents $BM^{0.07-0.12 [0.03; 0.20]}$) and PGLS analyses ($P < 0.001$, $\lambda = 0.00$; scaling exponents $BM^{0.08-0.12 [0.04; 0.19]}$). $MRT_{solute}FS$ was not related to BM in GLS ($P = 0.18$) and tended towards significance in PGLS with a strong phylogenetic structure ($P = 0.08$; $\lambda = 0.92$; scaling exponent $BM^{0.09 [-0.01; 0.18]}$), indicating that closely related species have similar $MRT_{solute}FS$ values, independent of their BM.

Comparison with literature data from ruminants: 'digesta washing' in the forestomach

The $SF_{2mm/solute}FS$ differed between ruminants and camelids ($\chi^2 = 125$; $P < 0.001$) with significantly lower values in 'moose-type' ruminants as compared with 'cattle-type' ruminants and camelids ($P < 0.001$) and a trend towards camelids being lower than 'cattle-type' ruminants ($P = 0.084$) (Fig. 4A). Correspondingly, a GLM with $MRT_{2mm}FS$ as independent and $MRT_{solute}FS$ as dependent variable revealed significant influence of herbivore type ($P < 0.001$), with 'moose-type' ruminants having higher $MRT_{solute}FS$ than 'cattle-type' or camelids at a given $MRT_{2mm}FS$ (Table 3; Fig. 4B). In a PGLS model with the same variables but without herbivore type as cofactor, there was a significant phylogenetic structure in the dataset ($\lambda = 0.781$), indicating similar values among closely related species. The fit of the GLS model with herbivore types was better than the PGLS model (AIC: -8.0 vs. 11.2). Note that the camelids do not achieve the very high $SF_{2mm/solute}FS$ or short $MRT_{solute}FS$ of cattle or muskoxen (*Ovibos moschatus*) (Lechner et al. 2010).

Comparisons with literature data from ruminants and non-ruminant foregut fermenters: sorting mechanism

The $SF_{10/2mm}GIT$ and FS differed between ruminants, camelids and NRFF ($\chi^2 = 52.9 / 52.8$; $P < 0.001$) with significantly lower values (close to equality) in NRFF as compared with

ruminants and camelids ($P < 0.001$) and lower values in ruminants compared with camelids (GIT: $P = 0.048$; FS: $P = 0.029$; Fig. 5A). In a GLM with $MRT_{2mm}FS$ as independent and $MRT_{10mm}FS$ as dependent variable, there was a significant interaction with herbivore type, irrespective of whether NRFF were included in the analyses ($P < 0.001$) or not ($P = 0.03$) (Table 4). While the significant interaction does not allow interpreting the exclusive effect of herbivore type in this relationship, the data analysis confirms that there is no particle sorting in NRFF, and that camelids are generally within the higher range of ruminants (Fig. 5B).

More data could be included in the comparison of $SF_{20/2mm}FS$ between herbivore types than for $SF_{10/2mm}FS$ ($n = 102$ vs. 85 datapoints), but no data from NRFF was available for $SF_{20/2mm}FS$. The $SF_{20/2mm}FS$ was lower in ruminants than in camelids ($T = 4.5$; $P < 0.001$; Fig. 6A). Again, in a GLS with $MRT_{2mm}FS$ as independent and $MRT_{20mm}FS$ as dependent variable, there was a significant interaction with herbivore type ($P = 0.001$). The data indicate that camelids are generally within the higher range of ruminants in this relationship (Fig. 6B).

In contrast, the $SF_{20/10mm}GIT$ and FS did not differ between ruminants and camelids ($W = 178 / 177$; $P = 0.49 / 0.47$) and was close to equality (Fig. 7A). Also, in a GLS with $MRT_{20mm}FS$ as independent and $MRT_{10mm}FS$ as dependent variable, there was no difference between camelids and ruminants ($P = 0.63$) (Fig. 7B).

In GLS models with $SF_{10/2mm}FS$ or $SF_{20/2mm}FS$ as independent variable and rDMI as dependent variable, the latter was not significant ($P > 0.10$), while there was again a significant difference between ruminants and camelids ($P < 0.001$), indicating generally higher values in camelids compared with ruminants, independent of food intake. Applying the same model for $SF_{20/10mm}FS$ revealed again no influence of rDMI ($P = 0.22$), but no difference between ruminants and camelids ($P = 0.67$). There were no significant interactions between rDMI and herbivore type in these models ($P > 0.11$). Note that the ranges of rDMI were overlapping for camelids and ruminants, but the range of rDMI data of the camelids were less

broad (27 to 44 g kg BM^{-0.85} d⁻¹) than the range of rDMI values from ruminants (8 to 107 g kg BM^{-0.85} d⁻¹).

Discussion

Differences between camelid species

In general, the absolute MRTs obtained from the camelids investigated in the present study do not confirm the particularly long retention times measured in other studies. For example, in Bactrian camels the MRTs measured in the GIT by Cahill and McBride (1995) were 50 to 80 % longer than the ones measured in the present study (MRT_{solute}GIT: 50 vs. 34 h; MRT_{2mm}GIT: 85 vs. 47 h). In the llamas, MRT_{solute}GIT and MRT_{2mm}GIT data from Heller et al. (1986a) exceeded the ones measured in the present study by approximately 30 %. These differences might well be explained by differences in the food intake level, as described for Bactrian camels by Cahill and McBride (1995), which influence MRT within and across species (Müller et al. 2013; Clauss et al. 2014). Therefore, measurements of retention time should ideally always be accompanied by assessments of intake, and conclusions made from comparisons of absolute MRTs must account for the effect of food intake (Levey and Martínez del Río 1999).

It would have been preferable to also include dromedaries into the experiment and feed them the same lucerne-based diet, but this was not possible due to a lack of available animals. No data on food intake or MRT_{10mm} were available for dromedaries in the literature, which is why dromedaries could not be included in the statistical comparisons between herbivores with respect to this marker. Generally, dromedary data from Lechner-Doll et al. (1990) indicated longer retention times in the GIT compared with Bactrian camels. Dromedaries had relatively short MRT_{solute}FS of 11 ±1 h compared with 19 ±3 h in Bactrian camels, and also shorter MRT_{solute}FS than the smaller species investigated in this study. MRT_{particle}FS were similar in dromedaries when compared with the camelids investigated in this study, which resulted in

comparably higher SF values in the FS of dromedaries (also documented by Heller et al. 1986c). Other measurements reported for tulus (hybrids of *C. bactrianus* and *C. dromedarius*) under hydrated conditions indicated short $MRT_{solute}FS$ of 12 h (von Engelhardt et al. 2006b), similar to the $MRT_{solute}FS$ in the dromedaries. Contrasting to the short $MRT_{solute}FS$ found in the camelids, long $MRT_{solute}FS$ in ruminants have been interpreted as a consequence of a proportionately large FS that serves as a water storage organ (Silanikove 1994; Hummel et al. 2008). Dromedaries, with a comparatively smaller proportionate FS volume than ruminants (Lechner-Doll et al. 1990), apparently do not use the FS to the same extent as a water reservoir. Actually, even after severe dehydration and a sudden re-hydration, the camelid FS does not maintain an enlarged volume for more than one day (von Engelhardt et al. 2006b). Therefore, the camelids' adaptation to water shortage appears to consist in the ability to rapidly ingest large amounts of water when it is available, and to absorb this water quickly into the body, rather than retain it in the FS (von Engelhardt et al. 2006b), as for example observed in the desert-adapted addax antelope (*Addax nasomaculatus*) (Hummel et al. 2008).

Comparing digesta washing between camelids and ruminants

Digesta washing can be described by the difference between the $MRT_{particle}$ and the MRT_{solute} , expressed as the ratio of the two measures, the SF. In the case of high ratios, a faster-moving fluid phase washes through a slower-moving particulate digesta phase, thereby removing solutes and very fine particles, including microbes, from this plug (Lentle et al. 2006; Müller et al. 2011). This process is not restricted to ruminants as it can also be found in some NRFF and other digestion types (Müller et al. 2011). Within ruminants, species differ in rumen fluid throughput and the degree of digesta washing (Clauss and Lechner-Doll 2001; Clauss et al. 2006; Dittmann et al. 2015; Hummel et al. 2015), which led to the classification of 'cattle-' and 'moose-type' ruminants. Therefore, the finding that 'cattle-' and 'moose-type' ruminants differ significantly in the $SF_{2mm/solute}FS$ (Fig. 4A) is no surprise, because the measure is

actually used for the classification. Therefore, it appears that camelids in general have evolved a 'cattle-type' strategy, although guanacos (*Lama guanicoe*) and vicuñas (*Vicugna vicugna*) have not yet been subjected to digesta retention measurements.

The proposed major advantage of the 'cattle-type' strategy is an increased harvest of microbes from the FS, leading to a higher general yield of microbial protein, and selection for a fast-growing and particularly efficient microbial community in the FS (Clauss et al. 2010; Dittmann et al. 2015; Hummel et al. 2015). Due to this higher microbial yield, the 'cattle-type' strategy might be particularly suitable for camelids with their greater ability to recycle urea as compared with domestic ruminants (Hinderer and von Engelhardt 1975; von Engelhardt and Schneider 1977). The 'moose-type' strategy has been linked with browse feeding and salivary defences against tannins (Hofmann et al. 2008; Codron and Clauss 2010). Because browse often represents the main component of the diet of free-ranging dromedaries (reviewed in Iqbal and Khan 2001), the 'cattle-type' dromedaries must have evolved alternative strategies to deal with tannins that are not related to saliva viscosity.

Considering only ruminants, the 'moose-type' strategy is prominent in basal groups such as the tragulids or giraffids (Clauss and Lechner-Doll 2001; Hummel et al. 2005; Darlis et al. 2012) and could therefore appear as the basal physiological strategy of the ruminant suborder. However, the high $SF_{2mm/solute}^{FS}$ in the more distantly related camelids could allow the interpretation that a higher degree of digesta washing as in 'cattle-type' ruminants represents the basal situation, and that the 'moose-type' strategy may be a more derived state. Although some evidence matches the latter hypothesis, e.g. the observation of the 'moose-type' strategy in the subfamily of the Cephalophines (Clauss et al. 2011) or in dikdik (*Madoqua* spp.) (Hebel et al. 2011), which are considered derived ecomorphs (Bärmann 2014), more measurements in a larger number of species are required to confirm this concept.

Comparing particle sorting in camelids, ruminants and non-ruminant foregut fermenters

The sorting of large versus small particles is crucial for the process of rumination, as it ensures that only those particles that can be efficiently further reduced in size are subjected to repeated mastication (Lauper et al. 2013). However, the actual sorting is rather based on particle density than on particle size (Baumont and Deswysen 1991; Lechner-Doll et al. 1991), because larger particles typically have a lower functional density, and hence a propensity to float in a liquid medium (Sutherland 1988; Clauss et al. 2009b). In the FS of ruminants, there is a clear distinction between the reticulorumen on the one hand, where particles of all sizes occur, and the omasum on the other hand, where only small particles are present (Clauss et al. 2009a; Clauss et al. 2009b). This means that the orifice between the reticulum and the omasum is a point of demarcation. In the FS of the camelids, however, this separation is somewhat less distinct. Although there is also a clear difference in particle sizes present between compartments C1/C2 (corresponding to the reticulorumen) and the distal part of C3/hindstomach (corresponding to the abomasum), there apparently is a more gradual transition within the proximal part of the C3 compartment, where not as many large particles as in C1/C2, but still more than in the distal C3, are present (Lechner-Doll and von Engelhardt 1989). This suggests that the orifice between C2 and C3, although similar in its width to that of the orifice between the reticulum and omasum in ruminants (Langer 1988), may not represent an absolute demarcation point in camelids. The similar, comparatively low faecal particle sizes in ruminants and camelids (Fritz et al. 2009) leads to the assumption that the large particles in C3 must be transferred back to the more proximal parts of the FS to be ruminated and thereby eventually reduced in size.

Comparing the findings on the retention of different-sized particles in camelids to those from ruminants reveals several similarities. The marker excretion curves recorded in the present study are generally similar to those found in ruminants (cf. e.g. our camelid Fig. 2 to the excretion curves shown in Schwarm et al. 2008 or Lechner et al. 2010). As previously found in ruminants, camelids also do not discriminate between particles of 10 or 20 mm. In

433 ruminants this pattern is independent of whether the markers are fed directly to the animal
434 (Schwarm et al. 2009a) or inserted into the rumen via fistula (Lechner et al. 2010), i.e. is not
435 affected by ingestive mastication. Therefore, it appears unlikely that the lack of discrimination
436 between these sizes is due to the method of marker application in the present study, i.e. that
437 large particle markers had been significantly reduced in size by mastication before they
438 reached the FS. Evidently, within species for which such data are available (llamas, Bactrian
439 camels, reindeer, muskoxen, moose, and cattle), particle size has no additional influence on
440 particle retention above a certain threshold of about 1 cm. Whether a similar threshold exists
441 in smaller species remains to be investigated.

442 The present comparison of relationships of large to small particle retention between
443 camelids, ruminants and NRFF suggests that a sorting mechanism sets ruminants and
444 camelids apart from other foregut fermenters (Fig. 5) and represents, given the distant
445 relatedness of camelids and ruminants, a convergent adaptation where the same function is
446 achieved by different morphophysiological designs. This convergence not only manifests in
447 patterns of MRT (present study), FS motility and chewing activity (Heller et al. 1986b; von
448 Engelhardt et al. 2006a), but also in particle size reduction (Fritz et al. 2009) and the high
449 fibre-digestibility when compared with other NRFF (Hintz et al. 1973; Sponheimer et al.
450 2003; Clauss et al. 2009c; Steuer et al. 2013).

451 The results of the present study indicate a quantitative difference in the sorting of large
452 vs. small particles between herbivore types, with longer 10 mm or 20 mm to 2 mm particle
453 retention in camelids as compared with ruminants (and NRFF), evident as higher
454 $SF_{10mm/2mm}^{FS}$ and $SF_{20mm/2mm}^{FS}$ (Fig. 5 and 6). These higher SF values appear to be caused
455 by a longer retention of large particles, rather than a shorter retention of 2 mm particles. The
456 difference between ruminants and camelids was not explained by differences in food intake
457 level, and hence might reflect true functional differences between the morphophysiological
458 designs of the ruminant and the camelid FS. Whether longer retention of large particles could

explain the observation that, under similar experimental conditions, camelids usually have a lower food intake than ruminants (Meyer et al. 2010; Dittmann et al. 2014a) and a generally lower level of metabolism (Dittmann et al. 2014a) remains speculative. Interpreting the effects of morphophysiological characteristics of the GIT as constraint for other physiological functions, and ultimately for the competitiveness and diversity of taxonomic groups, could lead to instructive narratives (e.g. Janis et al. 1994; Clauss and Rössner 2014). In the case of camelids, both more functional measurements, such as particle size distributions in the different FS compartments, and a systematic evaluation of the fossil record in comparison to ruminants, are necessary to support such a narrative.

Conclusion

The results of this study indicate a distinct convergence between camelids and ruminants in terms of the presence of a particle sorting mechanism in their digestive tracts, as well as in the degree of ‘digesta washing’ between camelids and ‘cattle-type’ ruminants. They also provide preliminary evidence that the particle sorting mechanism differs in detail between the two groups. To explore this putative difference, more detailed studies on the retention mechanism are required.

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Table 1 Sources for retention times measures of 2mm, 10mm, and 20mm particles in ruminants, camelids and non-ruminant foregut fermenters used in the comparative evaluation (see Dittmann et al. 2015 for a complete list of ruminant species with measurements for 2mm particles and solutes).

Species	Herbivore type	MRT _{2mm} GIT	MRT _{10mm} GIT	MRT _{20mm} GIT	MRT _{2mm} FS	MRT _{10mm} FS	MRT _{20mm} FS	MRT sources
<i>Cam. dromedarius</i>	Camelid	x		x	x		x	(Heller et al. 1986c; Lechner-Doll et al. 1990)
<i>Lama glama</i>	Camelid	x		x	x		x	(Heller et al. 1986a)
<i>Alces alces</i>	Ruminant	x	x	x	x	x	x	(Lechner et al. 2010)
<i>Bos javanicus</i>	Ruminant	x	x		x	x		(Schwarm et al. 2008)
<i>Bos taurus</i>	Ruminant	x	x	x	x	x	x	(Lirette and Milligan 1989; Lechner-Doll et al. 1990; Lechner et al. 2010)
<i>Capra hircus</i>	Ruminant	x		x	x		x	(Lechner-Doll et al. 1990)
<i>Ovibos moschatus</i>	Ruminant	x	x	x	x	x	x	(Lechner et al. 2010)
<i>Ovis aries</i>	Ruminant	x		x	x		x	(Lechner-Doll et al. 1990)
<i>Rangifer tarandus</i>	Ruminant	x	x	x	x	x	x	(Lechner et al. 2010)
<i>Tayassu tajacu</i>	NRFF	x	x		x	x		(Schwarm et al. 2009c)
<i>Hexapr. liberiensis</i>	NRFF	x	x		x	x		(Clauss et al. 2004; Schwarm et al. 2008)
<i>Hippop. amphibius</i>	NRFF	x	x		x	x		(Clauss et al. 2004)
<i>Colobus angolensis</i>	NRFF	x	x		x	x		(Schwarm et al. 2009c)
<i>Colobus polykomos</i>	NRFF	x	x		x	x		(Schwarm et al. 2009c)
<i>Presbytis johnii</i>	NRFF	x	x		x	x		(Schwarm et al. 2009c)
<i>Macropus rufus</i>	NRFF	x	x		x	x		(Schwarm et al. 2009c)

MRT mean retention time; GIT gastrointestinal tract; FS forestomach; NRFF non-ruminant foregut fermenter. Different data subsets have different numbers of species depending on whether information on dry matter intake and various retention measures were available

712 **Table 2** Body mass, dry matter intake, and retention times and selectivity factors of the gastrointestinal tract from the camelids investigated in this
713 study and from dromedaries (*C. dromedarius*) investigated by Lechner-Doll et al. (1990).

Species	Body mass (kg)	rDMI (g d ⁻¹ kg ^{-0.85})	----- MRT GIT (h) -----				----- SF GIT -----			
			Solute	2mm	10mm	20mm	2mm/solute	10/2mm	20/2mm	20/10mm
<i>Vicugna pacos</i>	63 ± 12	34 ± 7	34 ± 6 ¹	50 ± 12 ²	59 ± 12 ³	n.m.	1.46 ± 0.13	1.20 ± 0.14 ^a	n.m.	n.m.
<i>Lama glama</i>	148 ± 26	35 ± 5	29 ± 2 ¹	40 ± 4 ²	58 ± 4 ³	57 ± 3 ^{a3}	1.40 ± 0.09	1.47 ± 0.14 ^b	1.44 ± 0.11	0.98 ± 0.03
<i>Camelus bactrianus</i>	658 ± 72	35 ± 6	34 ± 3 ¹	47 ± 6 ²	66 ± 5 ³	67 ± 5 ^{b3}	1.38 ± 0.11	1.42 ± 0.07 ^b	1.43 ± 0.07	1.01 ± 0.02
<i>Camelus dromedarius</i>	453 ± 95	n.m.	45 ± 8*	61 ± 7*	n.m.	88 ± 12*	1.36 ± 0.09	n.m.	1.43 ± 0.10	n.m.

714 rDMI relative dry matter intake; MRT mean retention time; GIT gastrointestinal tract; SF selectivity factor; n.m. not measured; superscript letters indicate significant differences
715 between MRT measures and SFs within columns, superscript numbers indicate differences of MRT or SFs within species, asterisks indicate significant differences in the
716 respective means between *C. bactrianus* and *C. dromedarius*.
717

Table 3 Retention times and selectivity factors of the forestomach from the camelids investigated in this study and from dromedaries (*C. dromedarius*) investigated in Lechner-Doll et al. (1990).

Species	----- MRT FS (h) -----				----- SF FS -----			
	Solute	2mm	10mm	20mm	2mm/solute	10/2mm	20/2mm	20/10mm
<i>Vicugna pacos</i>	22 ± 7^1	38 ± 11^2	47 ± 11^3	n.m.	1.74 ± 0.26	1.28 ± 0.22^a	n.m.	n.m.
<i>Lama glama</i>	17 ± 3^1	28 ± 3^2	47 ± 5^3	45 ± 4^{a3}	1.71 ± 0.23	1.66 ± 0.15^b	1.62 ± 0.12	0.97 ± 0.05
<i>Camelus bactrianus</i>	19 ± 3^1	32 ± 5^2	51 ± 4^3	51 ± 4^{b3}	1.72 ± 0.29	1.63 ± 0.14^b	1.64 ± 0.11	1.01 ± 0.04
<i>Camelus dromedarius</i>	$11 \pm 1^*$	$26 \pm 3^{(*)}$	n.m.	53 ± 7	$2.52 \pm 0.31^*$	n.m.	$2.01 \pm 0.30^*$	n.m.

n.m. not measured; MRT mean retention time; FS forestomach; SF selectivity factor; superscript letters indicate significant differences between MRT measures and SF within columns, superscript numbers indicate differences of MRT within species, asterisks indicate significant differences in the respective value between *C. bactrianus* and *C. dromedarius*, while asterisks in brackets indicate trends.

724 **Table 4** Linear regression equations corresponding to $\log(y) = a + b \log(x) + \text{Cofactor}$, including the interaction of cofactor $\times \log(x)$ if significant.

725 PGLS was carried out without the cofactor, instead lambda (λ) was calculated.

Model	Variables			n	Intercept			Independent variable			Cofactor		Interaction		AIC
	y	x	Cofactor		a	T	p	b	T	p	F	p	F	p	
GLS	MRT _{solute} FS	MRT _{2mm} FS	Cam/Rum	35	3.3	1.11	0.278	0.65	5.13	< 0.001	0.17	0.682	-	ns	16.7
GLS	MRT _{solute} FS	MRT _{2mm} FS	Cam/Moose/Cattle	35	0.1	-2.14	0.041	1.04	9.48	< 0.001	17.85	< 0.001	-	ns	-8.0
PGLS	MRT _{solute} FS	MRT _{2mm} FS	- ($\lambda=0.78$)	35	4.3	1.38	0.176	0.65	5.40	< 0.001	-		-	-	11.2
GLS	MRT _{10mm} FS	MRT _{2mm} FS	Cam/Rum	54	225.0	4.50	< 0.001	0.44	2.90	0.006	5.75	0.020	4.96	0.031	-
GLS	MRT _{10mm} FS	MRT _{2mm} FS	Cam/Rum/NRFF	85	225.0	4.61	< 0.001	0.44	2.97	0.004	14.68	< 0.001	8.72	< 0.001	-
GLS	MRT _{20mm} FS	MRT _{2mm} FS	Cam/Rum	102	2968.9	7.94	< 0.001	0.13	0.99	0.325	12.14	0.001	11.32	0.001	-
GLS	MRT _{20mm} FS	MRT _{10mm} FS	Cam/Rum	39	2.2	3.65	0.001	0.91	38.42	< 0.001	0.53	0.470	-	ns	-

726 GLS, general least squares; PGLS, phylogenetically informed GLS; MRT, mean retention time; FS, forestomach; Cam, camelids; Rum, ruminants; Moose, 'moose-type'
 727 ruminants; Cattle, 'cattle-type' ruminants; NRFF, non-ruminant foregut fermenters; AIC, Akaike information criterion.

Figure Legends

Figure 1 Schematic comparison of the morphology of the forestomach complex (viewed from its left side, with parts that cannot be viewed from the left displaced underneath) in A) ruminants and B) camelids (Lechner-Doll et al. 1995). The ruminant forestomach consists of the rumen (with various sub-compartments), the reticulum, the omasum and the abomasum. The reticulum and omasum are linked by the reticulo-omasal orifice. The camelid forestomach consists of the first compartment (C1, with a cranial and a caudal sub-compartment and typical ‘glandular sacs’), the second compartment (C2, also sometimes referred to as the ‘reticulum’, also containing ‘glandular sacs’), and a third compartment (C3, consisting of a cranial part and a caudal ‘hindstomach’). The C2 and the C3 are linked by a small tubular canal. White surfaces represent a stratified epithelium (in the case of ruminants, with papillae in the rumen and the typical honeycomb cells in the reticulum), dotted areas represent cardiac glands (covering the ‘glandular sacs’ and the cranial portion of C3), and striped areas represent acid-secreting glandular stomach epithelium.

Figure 2 Exemplary excretion curves of the solute and particle markers of each one alpaca (*Vicugna pacos*), llama (*Llama glama*), and Bactrian camel (*Camelus bactrianus*).

Figure 3 Relationships of (A) the mean retention time of 2 mm particles in the gastrointestinal tract (MRT_{2mmGIT}) and (B) the mean retention time of solutes in the gastrointestinal tract ($MRT_{soluteGIT}$) with body mass (BM) in ruminants and camelids. Dots represent species means.

Figure 4 (A) Comparison of the $SF_{2mm/solute}FS$ between species means from camelids and ruminants (‘cattle-‘ or ‘moose-type’). Boxplots indicate median, upper and lower quartile, as well as maximum and minimum values, dots indicate outliers, asterisks represent significant

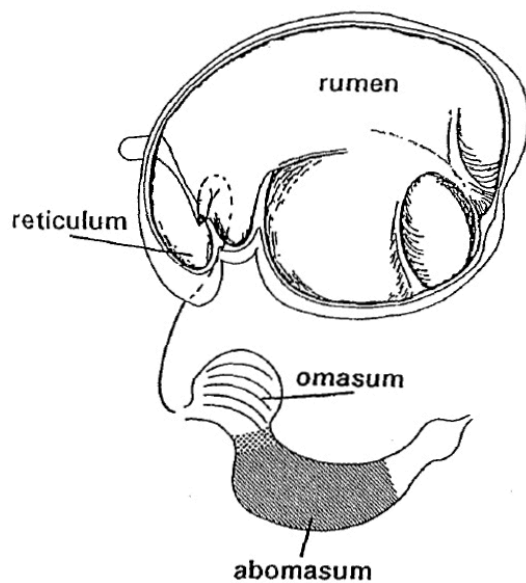
differences between herbivore types ($P < 0.05$). (B) Relationship between $MRT_{\text{solute}}\text{FS}$ and $MRT_{2\text{mm}}\text{FS}$ in ruminants and camelids; dots represent species means; the dashed line represents equality of the two measures, i.e. an SF of 1.

Figure 5 (A) Comparison of individual data of the $SF_{10/2\text{mm}}\text{FS}$ between camelids (Cam), Ruminants (Rum), and non-ruminant foregut fermenters (NRFF). (B) Relationship between $MRT_{10\text{mm}}\text{FS}$ and $MRT_{2\text{mm}}\text{FS}$ in the same herbivores; dots represent measurements of individuals; the dashed line represents equality of the two measures, i.e. an SF of 1.

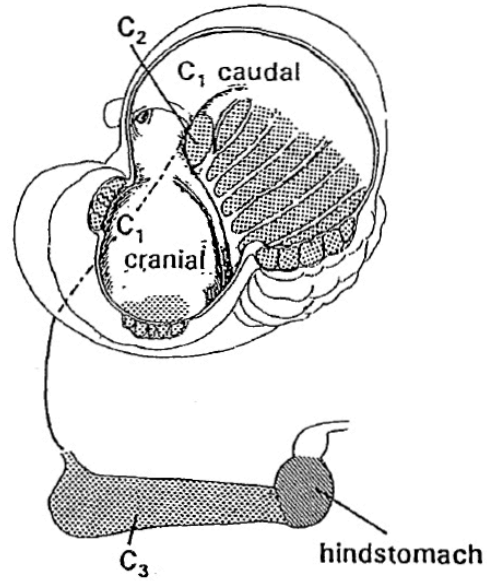
Figure 6 (A) Comparison of individual data of the $SF_{20/2\text{mm}}\text{FS}$ between camelids (Cam) and ruminants (Rum). (B) Relationship between $MRT_{20\text{mm}}\text{FS}$ and $MRT_{2\text{mm}}\text{FS}$ in ruminants and camelids; dots represent measurements of individuals; the dashed line represents equality of the two measures, i.e. an SF of 1.

Figure 7 (A) Comparison of individual data of the $SF_{20/10\text{mm}}\text{FS}$ between camelids (Cam) and ruminants (Rum). (B) Relationship between $MRT_{20\text{mm}}\text{FS}$ and $MRT_{10\text{mm}}\text{FS}$ in ruminants and camelids; dots represent measurements of individuals; the dashed line represents equality of the two measures, i.e. an SF of 1.

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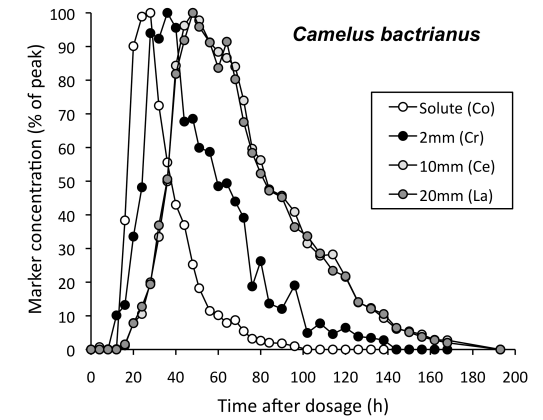
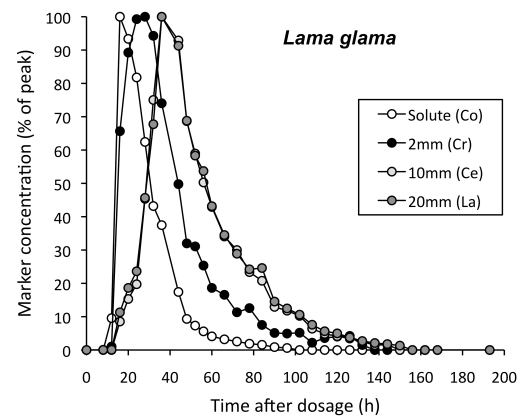
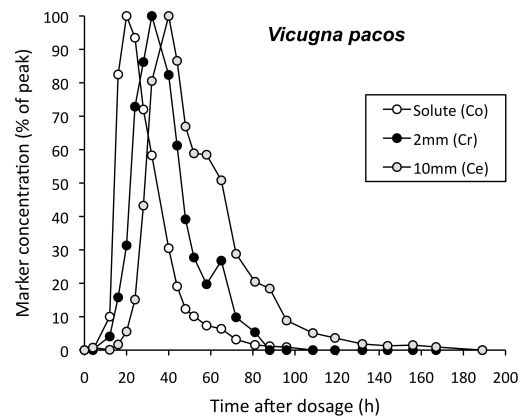
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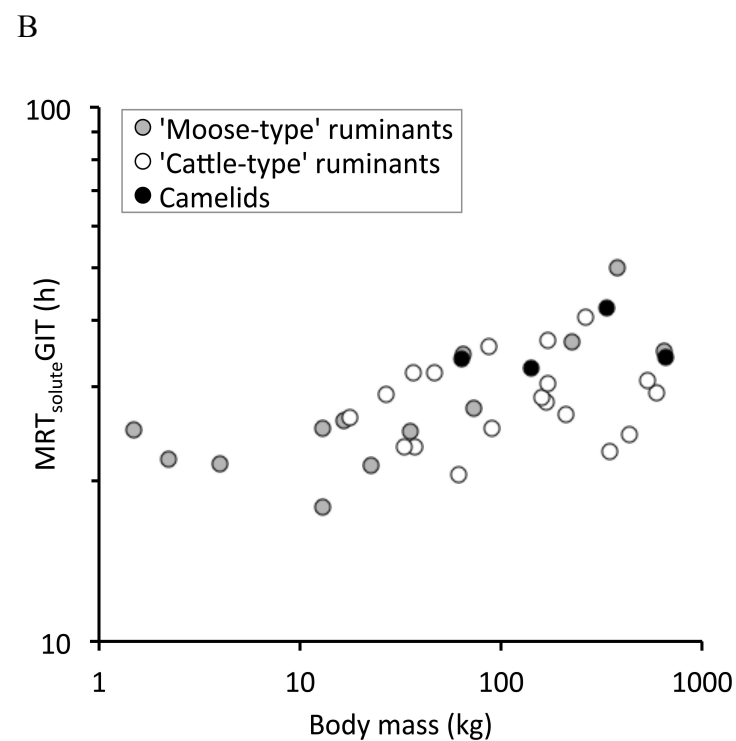
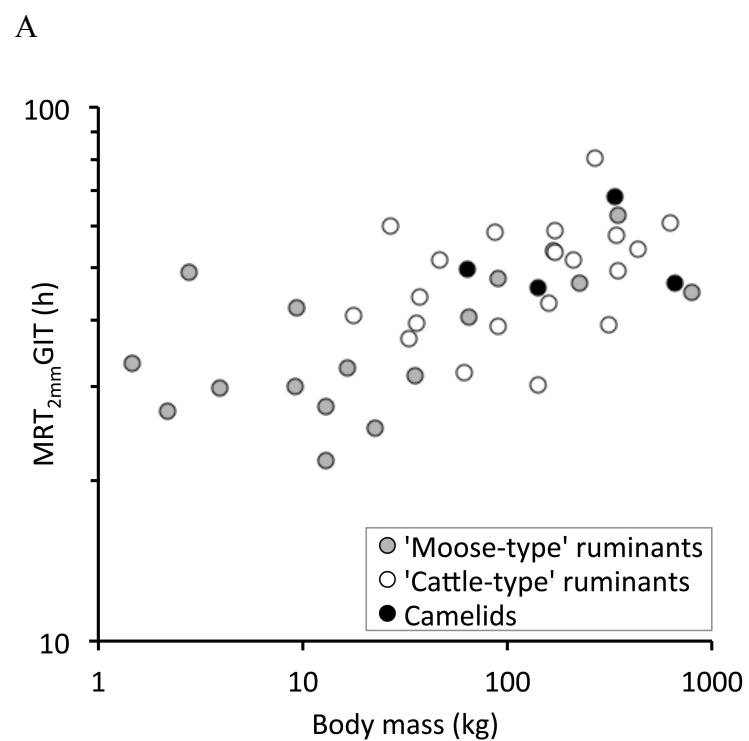
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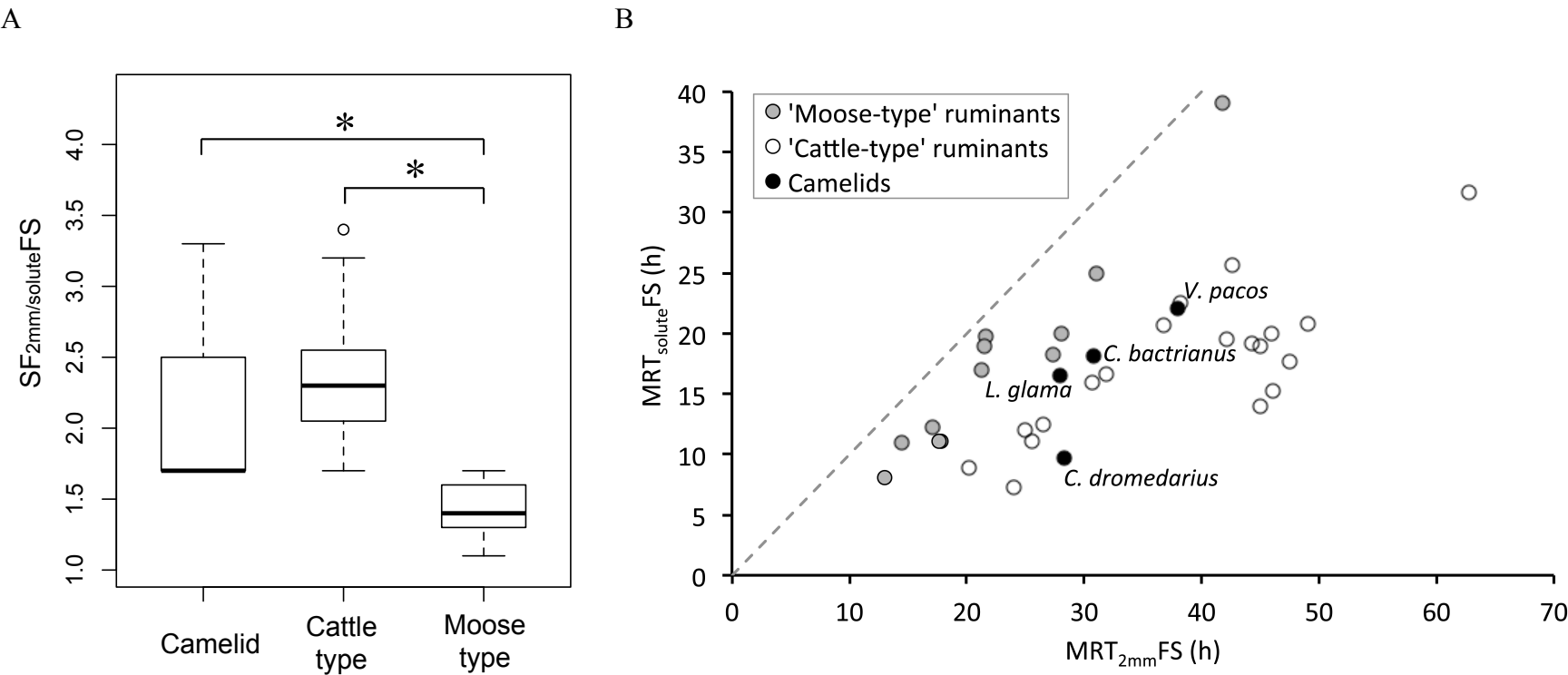


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792 **Figure 3** Relationships of (A) the mean retention time of 2 mm particles in the gastrointestinal tract (MRT_{2mm}GIT) and (B) the mean retention time
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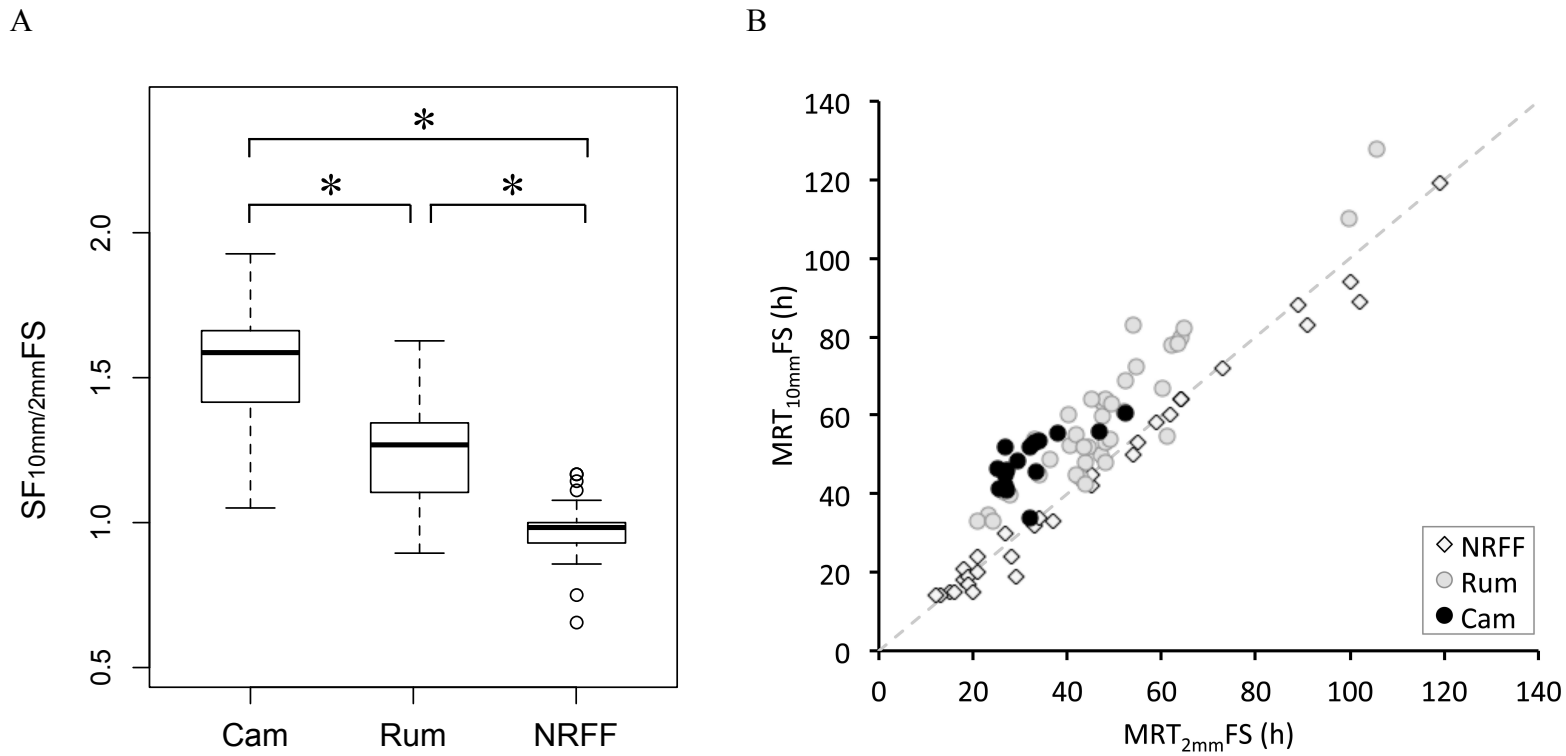
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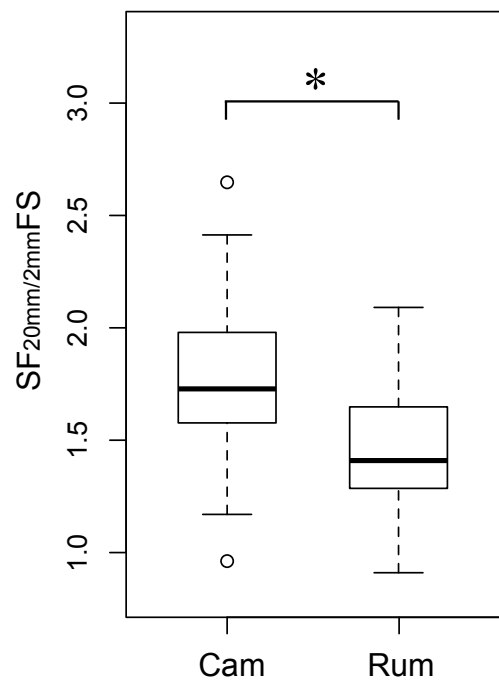
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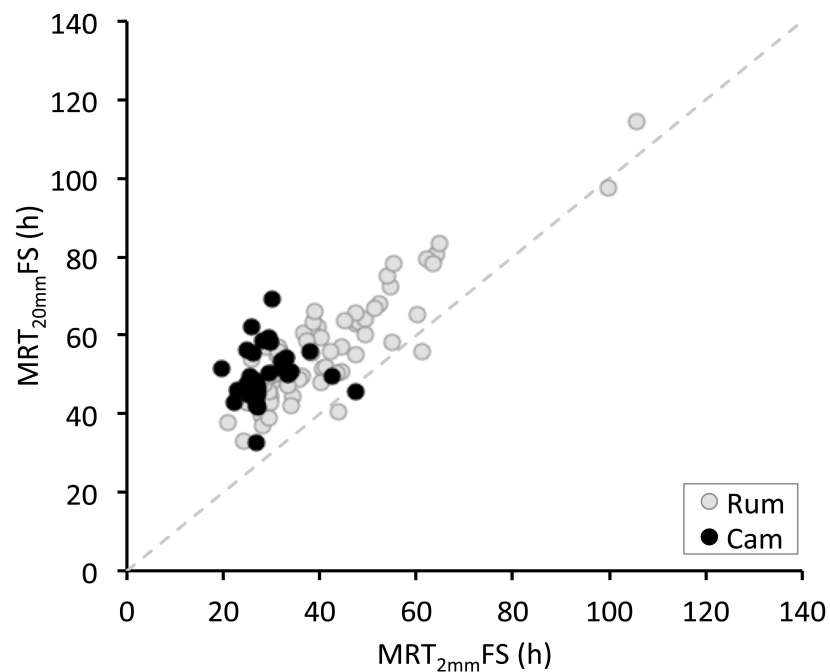
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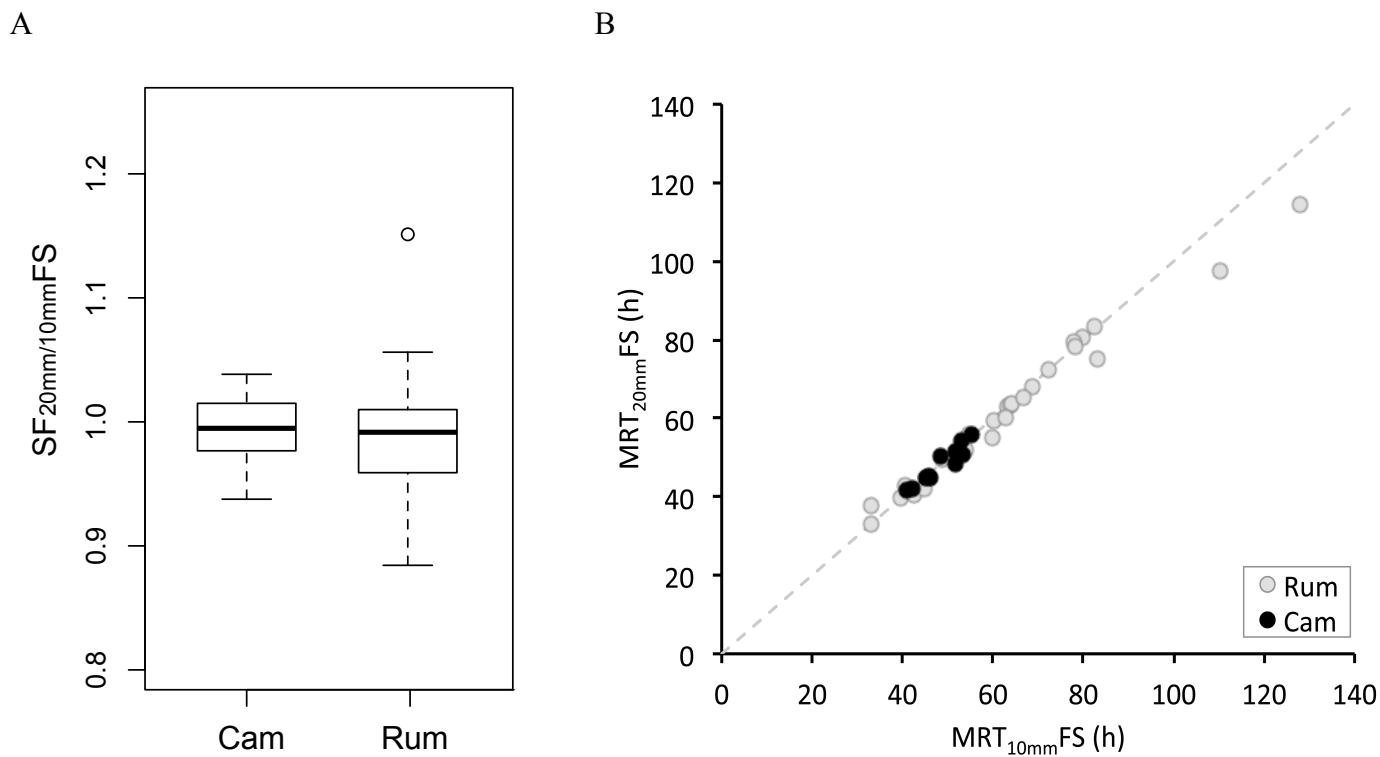
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